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Neuro protective Study of *Withania somnifera Dunn.* by an *In Silico* Approach

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A B S T R A C T

Parkinson's disease is a progressive neurodegenerative condition resulting from the death of the dopamine containing cells of the substantia nigra. In this present study the neuroprotection was studied by root tuber ethanolic extracted compounds of *Withania somnifera Dunn.* to inhibit the protein DJ1 by using *in silico* approach. The structures of ligands were downloaded from pubchem and converted into pdb files in docking server. The docking scores of DJ1 with Episilon cadinene, Oleic acid, Pyrogallol, 2, 3 dihydroxypropyl N- (8- (Trifluoromethyl)-4- Quinolyl) anthranilate, Pentadecanoic acid, showed significant interaction with active binding residues. The results of the present study may provide useful insights for developing new neuroprotective drugs. This information can be used for structure and pharmacophore based new drug designing for development of novel therapeutic agents for the prevention and treatment of Parkinson disease.

Keywords: Parkinson's disease, substantia nigra, *Withania somnifera*, DJ1 Protein, Pubchem.

A R T I C L E I N F O

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1. Introduction

Neurodegeneration refers to a condition of neuronal cell death occurring as a result of progressive disease of long term. It involves degeneration of circumscribed group of neurons that may be functionally or neuroanatomically connected (Manish *et al*., 2011). Parkinson's disease (PD) is a progressive neurodegenerative condition resulting from the death of the dopamine containing cells of the substantia nigra. By the time of death, this region of the brain has lost

50–70% of its neurons compared with the same region in unaffected individuals (Davie C.A., 2008). DJ1 (PARK7), a gene on chromosome 1p36, has been associated with early-onset of PD, although the age of onset due to a mutation at these loci is currently contended. The DJ1 protein is found in the mitochondrial matrix and the intermembrane space and is particularly abundant in the brain. It counteracts the effects of mitochondrial complex 1 inhibitors such as hydrogen peroxide (Gandhi *et al.,* 2005). Studies have shown that gene deletion, mutation or down-regulation of DJ1 leaves cells vulnerable to oxidative damage by the complex 1 inhibitors that are left unregulated (*Olzmann et al., 2007).* In this present study the Neuroprotective activity of *Withania somnifera Dunn*. plants was studied by in silico studies against DJ1Protein. *Withania somnifera Dunn*. (Solanaceae) also commonly known as ash wagandha is a well known herb in the ayurvedic and indigenous medical systems for 3000 years (Jaffer *et al*., 1988). The roots of the plant are categorized as rasayanas, which are reputed to promote health and longevity by augmenting defence against disease, arresting the ageing process, revitalising the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental wellbeing (Girdhari *et al.,* 2007).

2. Materials and Methods

Collection of Sample and preparation of sample for GC - MS Analysis: The *Withania somnifera Dunn.* fresh root samples were collected from the area of Adiannamalai, Thiruvannamalai District. The Fresh *Withania somnifera Dunn.* roots were cleaned with deionized water and dried at shade for a week. The dried plant samples were ground well into fine powder in mixer grinder. About 25 g of dried root powder samples were soaked with 100 ml Absolute alcohol for 2days. Then the extracts were evaporated. The plant residues obtained were used for GCMS analysis (Sentil kumar *et al*., 2011).

GC-MS Analysis

The GC-MS analysis of the *Withania somnifera Dunn.* plants root extract with in absolute alcohol, was performed using Jelo GC mate II and the column is HP5ms. High pure Helium was the carriers gas at a flow rate of 1 ml/min the injector was operated at 220°C and the oven temperature was programmed as follows; 50°C to 250°C at 10 min. The scan range is 50 to 600 amu. The identification of components was based on comparison of their mass spectra with those of Wiley and NIST Libraries and those described by Adams.

Molecular docking studies of *Withania somnifera Dunn.* compound against Parkinson's disease

The compounds obtained from GC-MS study of *Withania somnifara* ethanolic extract were considered as ligands for the present docking study of DJ1. The structures of ligands were downloaded from pubchem and converted into pdb files in docking server.

PARK 7: The PARK 7 gene which encodes the protein DJ1 was retrieved from Protein Data Bank (PDB) with PDB id code 3EZG. The active sites of protein DJ1 were identified by Docking Server.

Molecular Docking

The docking of ligands into DJ1 was carried using Docking server (http://www.dockingserver.com/) Gasteiger partial charges were added to the ligand atoms. Non polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on ligand*s* and protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (Morris and Goodsell *et al.,* 1998). Auto Dock parameter set and distance dependent dielectric functions were used in the calculation of the vander Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (Solis and Wets, 1981). Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 50 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

Ligands 3D structure generation

From the GC - MS study, ethanolic extracts of the root tubers of *Withania Sominfera,* the highest peak showing active compounds were selected as ligands for the docking study (Table 1). Their structures were directly downloaded from pubchem at NCBI.

Protein preparation

KEGG pathway is used to identify the inhibiting activity of DJ1 may effective reduce the Parkinson's disease symptoms. The PARK 7 gene which encodes the protein DJ1 was observed and retrieved from Protein Data Bank (PDB) (http://www.rcsb.org/ pdb/home/home.do). The active sites of protein DJ1 were identified by Docking Server.

Optimisation of protein

The protein DJ1 downloaded from PDB was prepared for docking by deleting all hetero atoms, ligands and water molecules and optimized by minimization of energy by using Docking server, an online tool.

3. Results and Discussion

GC- MS Analysis of *Withania somnifera*

The present study was carried out on *Withania somnifera Dunn.*root tuber extract with absolute alcohol, the phytochemical compounds were screened by GC-MS analysis. In the GC-MS analysis, 8 bioactive phytochemical compounds were identified in the extract of Withania *somnifera.* The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula. The results are presented in (Table 1) and chromatogram.

Molecular docking studies of *Withania somnifera Dunn.* plant compounds against Parkinson's disease

Initially, the 3D structure of DJ1 and 3D structure of ligands used in the study were downloaded in .pdb format DJ1 docking was performed with ligands using Docking

server. All these docking results reveal that, Episilon cadinene, Oleicacid, Pyrogallol, 2,3 dihydroxypropyl N- (8-(Trifluoromethyl)-4- Quinolyl) anthranilate, Pentadecanoic acid has good free energy of binding to hold enough in the active site and makes strong VdW interaction with DJ1. These results suggest that, these ligands are capable of inhibiting the DJ1.

Figure 1: Root tubers from *Withania somnifera Dunn.*

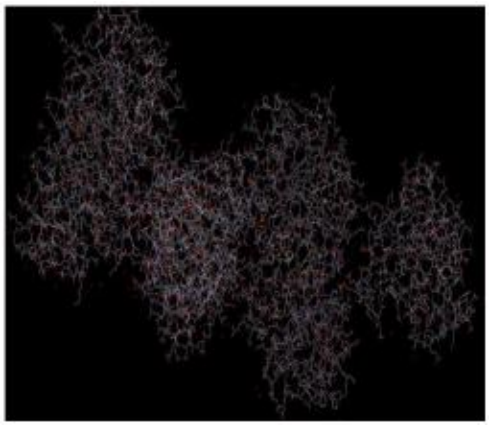


Figure 2: The DJ 1 3D Structure

Discussion

The neuroprotection is to limit neuronal dysfunction after injury and attempt to maintain the possible integrity of cellular interactions in the brain resulting in undisturbed neural function. There is a wide range of neuroprotection products available and some products can potentially be used in more than one disorder. These products may be of various kinds and can be classified as free radical scavengers, anti excitotoxic agents, apoptosis inhibitors, neurotrophic factors. Different aspects of neuroprotection are being examined, concentrating on different elements leading to loss of nerve cells. In this present study the *Withania somnifera Dunn.* was used to evaluate the Neuroprotective activity against Parkinson's disease (DJ1protein) studied by in silico approach. Episilon cadinene, Oleicacid, Pyrogallol, 2,3 dihydroxypropyl N-(8-(Trifluoromethyl)-4- Quinolyl) anthranilate, Pentadecanoic acid has good free energy of binding to hold enough in the active site and makes strong VdW interaction with DJ1.

The docking scores of DJ1 with these compounds showed significant interaction with active binding residues. The docking studies also revealed that 18: GLN, 76: ASN, 107: ALA, 128: LEU, 126: HIS, 15: GLU, 48: ARG, 80: GLN, 110: THR, 132: LYS residues of DJ1 protein form hydrogen bonds with the side chain along with main chain interaction with ligands name. The estimated free energy of bindings was -5.27 kcal/mol, -2.90 kcal/mol, -2.74 kcal/mol, -2.06 kcal/mol & -1.39 kcal/mol and estimated inhibition constant was 2.00 mM, 3.16 mM, 12.92 mM, 2.97 mM and 298.32 uM. The results of the present study may provide useful insights for developing new neuroprotective drugs. This information can be used for structure and pharmacophore based new drug designing for development of novel therapeutic agents for the prevention and treatment of Parkinson's disease.

Figure 3: Ligands

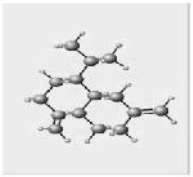
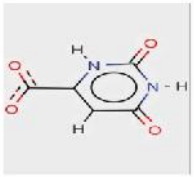
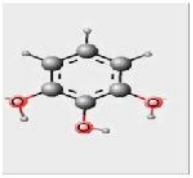
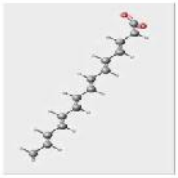
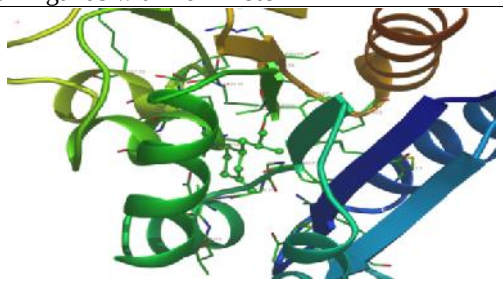
Compound Name	**Epsilon Cadinene**	**Oleic acid**	**Pyrogallol**	**Pentadecanoic acid**
3D Structure of Ligands				

Figure 4: Docked view of Ligands with DJ1 Protein

Epsilon Cadinene and DJ1	

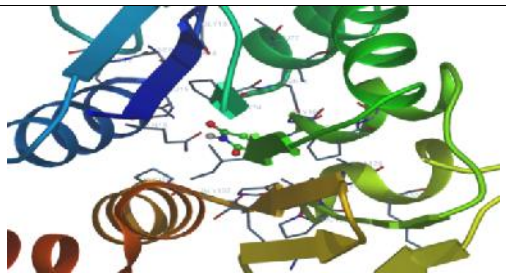
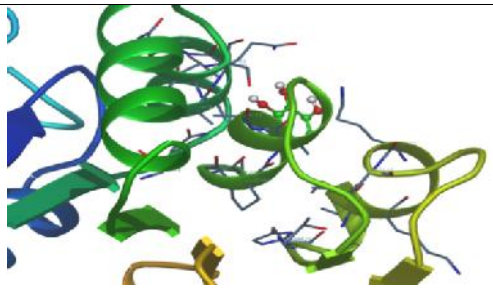
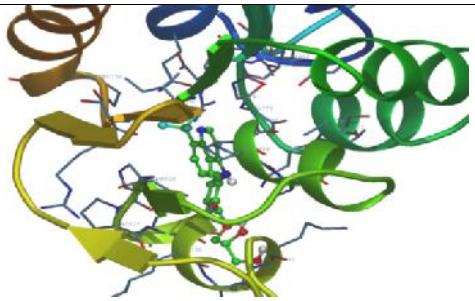
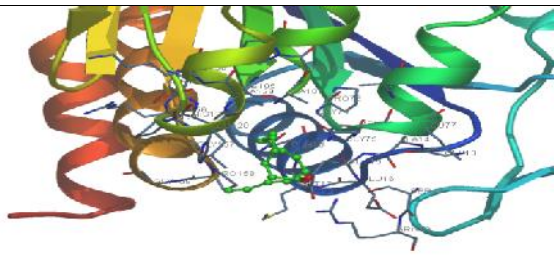
Oleic acid and DJ1	
Pyrogallol and DJ1	
2,3 dihydroxypropyl N- (8-(Trifluoromethyl)-4- Quinolyl) anthranilate and DJ1	
Pentadecanoic acid and DJ1	

Table 1: The identified phytochemical compounds of *Withania somnifera* using GC-MS analysis**.**

S.No	Name of the compound	Retention time	Molecular weight
1	Pentadecanoic acid	15.18	270.6424
2	14-methyl	15.15	270.6424
3	Methyl ester	15.15	270.6424
4	2,4-Imidazolidinedione	12.23	355.5102
5	5-(3,4-Bis(trimethylsilyl)-3-methyl-5-phenyl-1-trimethyl silyl	12.23	355.5102
6	Oleic acid	16.97	264.6356
7	Oleic acid	18.45	264.6244
8	Oleic acid	19.25	264.244
9	9-Octadecanoic acid	20.43	264.6356
10	Hexyl ester	20.43	264.6356
11	9-Octadecanoic acid	21.28	264.6356
12	2,3-Dihydroxy propylester	21.28	264.6356
13	9-Octadecanoic acid	23.82	264.6424
14	2,3-Dihydroxy propylester	23.82	264.6424

Table 2: Docking results table

Compound Name	Est. Free Energy of Binding	Est. Inhibition Constant. KI	Vdw + Hbond + dssolv Energy	Electrosatic Energy	Total Intermol. Energy	Frequency	Interact. Surface
Episilon cadinene	-5.27 kcal/mol	136.26um	-5.58 kcal/mol	+0.01 kcal/mol	-5.57 kcal/mol	100%	565.908
Oleicacid	-2.90 kcal/mol	7.42 mM	-3.15 kcal/mol	-0.05 kcal/mol	-3.29 kcal/mol	20%	30.125
Pyrogallol	-2.74 kcal/mol	9.83mM	-2.54 kcal/mol	-0.13 kcal/mol	-2.68 kcal/mol	80%	357.5
2,3 Dihydroxy propyl N- (8-(trifluoro methyl)-4- quinolyl) anthranilate	-2.06 kcal/mol	30.75mM	-4.48 kcal/mol	-0.14 kcal/mol	-4.62 kcal/mol	10%	581.694
Pentadecanoic acid	-1.39 kcal/mol	95.47 Mm	-4.81 kcal/mol	-0.01 kcal/mol	-4.82 kcal/mol	10%	614.922

Table 3: Docking calculation

Compound	Est. Free Energy of Binding
Episilon cadinene	-5.27 kcal/mol
Oleicacid	-2.90 kcal/mol
Pyrogallol	-2.74 kcal/mol
2,3 Dihydroxypropyl N- (8- (trifluoromethyl) -4- quinolyl)anthranilate	-2.06 kcal/mol
Pentadecanoic acid	-1.39 kcal/mol

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